



## REVIEW

on a dissertation work for obtaining the educational and scientific degree "doctor" in: field of higher education 4. Natural sciences, mathematics and informatics, professional direction 4.4 Earth sciences, scientific specialty "Ecology and preservation of ecosystems"

**Author of the dissertation:** Petya Georgieva Zaharieva, full-time doctoral student at the Department of "Agroecology and Environmental Protection" at the Agricultural University, Plovdiv

**Topic of the dissertation:** "Content of heavy metals in fish and their parasites from the Danube River - ecology and bioindication"

**Supervisor:**

Prof. Dr. Diana Kirin

**Reviewer:** Prof. Dr. Vasil Kostadinov Atanasov from the Faculty of Agriculture at Trakia University, Stara Zagora; Registered in NACID for the scientific degree "Doctor of Agricultural Sciences", in Professional direction 6.3. "Livestock" and "Professor" by field of higher education 4. Natural sciences, mathematics and informatics, Professional direction 4.3. "Biological Sciences", Scientific specialty "Biochemistry", designated as a member of the scientific jury by order No. RD-16-1117/31.10.2022. from the AU Rector.

### 1. Brief introduction of the candidate.

Doctoral student Petya Georgieva Zaharieva was born on March 29, 1991 in the city of Plovdiv. He completed his higher education in 2014. at the Agricultural University in his hometown, majoring in Agrarian Economics. In 2016 obtained a master's degree in the specialty "Ecology of settlement systems", professional qualification "Ecologist". Petya Zaharieva was educated in a doctoral program at the Department of "Agroecology and Environmental Protection", Agrarian University - Plovdiv, Faculty of Plant Protection and Agroecology in the scientific specialty: "Ecology and Protection of Ecosystems" in Professional direction 4.3 Biological

Sciences. The training was carried out in regular form during the period 2019-2022.

## **2. Relevance of the problem.**

The presented dissertation work is quite extensive - it is written on 250 pages and contains the standard structural components characteristic of this kind of development. The topic is particularly relevant, as it affects the content of heavy metals in fish and their parasites from the Danube River. The development is in line with the EU Strategy for the protection of biological diversity until 2030. It is also in sync with the National Strategy for Scientific Research in the Republic of Bulgaria 2017-2030, Specific Objective 1 for the development of the scientific career of young scientists. In this aspect, the development is in line with the main priority of Ecology - the protection of the environment, and hence human health. The main thesis of the development, that fish and their parasites can be used as ecological bioindicators, is supported by the applied scientific literature - 349 literary sources were used, of which 28 in Cyrillic and 285 in Latin, 6 normative documents, 10 standardized methods (BDS ) and 20 online databases. The extensive literature review of over 50 pages shows the doctoral student's excellent awareness of the problem, his in-depth knowledge and the great potential for solving important practical tasks in determining the content of heavy metals in fish and their parasites from the Danube River.

## **3. Purpose, tasks, hypotheses and research methods.**

The goal closely correlates with the title of the dissertation, and the set tasks logically form 9 work packages, the implementation of which is generally related to changes in the content of Cu, Cd and As in tissues and organs of fish, in helminth parasites of fish, as well as in waters and sediments. The object of the research is extremely suitable for proving the working hypotheses of the dissertation work, since the used carp fish (carp, bream and bream) as well as their parasites have the ability to accumulate significant amounts of heavy metals. The methods used are adequate to the research, and the modern equipment and methods are suitable for solving the identified tasks. Variational statistical processing of the data was used to determine a number of important dependencies.

## **4. Transparency and presentation of the obtained results.**

The dissertation is written in a good and understandable scientific style. It is very well illustrated with 42 tables and 106 figures. The "Results" section, in fact, also contains a discussion of the obtained data and its relatively skillful interpretation. A

general summary was made and 12 items were formulated. conclusions that give a complete picture of the dissertation work. Also presented are 12 scientific and scientific-applied contributions, as well as 8 valuable recommendations for ecology and fish farming and fishing practice.

### **5. Discussion of results and used literature.**

The dissertation student presents the obtained scientific and applied results in a very well-illustrated "Results" section. The present section presents the results of the studies on Cu, Cd and As content in liver, skin and muscles of dominant fish species of the Cyprinidae family (bream, bream and bream) from the freshwater ecosystem of the Danube River (Kudelin biotope), which differ in their way of life and diet. The logical sequence for solving the set tasks has been observed. A detailed picture of the tissue content (skin, muscle and liver) of Cu, Cd and As in the studied carp species and their parasites was made. The data on the content of Cu, Cd and As in waters and sediments from the Bulgarian section of the Danube River have been updated with the results from the Kudelin biotope. The seasonal and annual dynamics of the studied chemical elements in the liver, skin and muscles of the studied hydrobionts were determined. Interesting data were found on the values of the bioconcentration factor of Cu, Cd and As in liver, skin and muscles of fish compared to waters and sediments from the Danube River in the Bulgarian section. The bioindicative role of the liver, as a universal biochemical laboratory of the body with an increased metabolic load, is highlighted. The scientific literature has been enriched on the content of Cu, Cd and As in tissues and organs of bream, bream and bream and in some of their parasites, as well as in waters and sediments of the freshwater ecosystem of the Danube River. By calculating the values of the bioconcentration factor for the studied elements, it was concluded that for some of them a greater accumulation was observed in the parasites compared to the organism of the studied fish. According to the PhD student, the annual differences in the content of Cu, Cd and As in the liver, skin and muscles of the three fish species studied are due to the different content of the trace elements in water, sediments and in the food used. In accordance with what has been achieved, a relatively skillful interpretation has been made, including the research of world authors. In this section, the erudition of the author, the depth of entering the problem and the level of training achieved in the doctoral program are best seen. In this aspect, the aim and tasks of the dissertation work have been fulfilled, as a result of which 12 conclusions have been formulated. Valuable recommendations for the fish farming and fishing industry have been given regarding the suitability of certain organs for

consumption of the studied carp species. Additionally, emphasis is placed on the need to optimize cross-border cooperation in connection with improving the state of the freshwater ecosystem and preserving species diversity in the Danube River.

## **6. Contributions of the dissertation work.**

The contributions of each scientific work show its usefulness, both in fundamental and in scientific-applied aspect. In this sense, Petya Zaharieva's dissertation is a large-scale study, as a result of which 12 contributions are exposed in general, which could be briefly summarized and arranged as follows:

### **Scientific contributions**

□ For the first time in our country and in the Bulgarian section of the Danube River, a complex study was carried out on the content of Cu, Cd and As in the liver, skin and muscles of carp and its helminth parasites. Original scientific contribution for Bulgaria;

□ Data on bioconcentration factor values for Cu in liver, skin and muscle of sea urchin versus waters and sediments are reported for the first time; for Cd in liver, skin and muscle of bream versus water and sediment; for Cu in liver, skin and muscle of bream versus waters; for Cu, Cd, and As in liver, skin, and muscle of carp versus waters and sediments. Original scientific contribution;

□ For the first time, the contents of Cu, Cd and As in liver, skin and muscles of bream, bream and bream are compared. For the first time, the circulation of Cu and Cd in liver, skin and muscle of bream and bream, as well as the circulation of Cu, Cd and As in liver, skin and muscle of bream, their parasites, waters and sediments are examined. Original scientific contribution;

### **Scientific and applied contributions**

□ New data on the content of Cu in liver, skin and muscle of uklei are provided; of Cu and Cd in helminth parasites of uklei; of Cd in liver, skin and muscles of bream from the Danube River and the river basin in Bulgaria. Original scientific and applied contribution;

□ The data on the content of Cd and As in liver, skin and muscles of uklay have been updated; of Cu and As in liver, skin and muscle of bream; of Cu, Cd and As in waters and sediments from the Bulgarian section of the Danube - Biotope Kudelin. Original scientific and applied contribution;

□ For the first time, the seasonal and annual changes in the content of Cu and Cd in the liver, skin and muscles of bream and the seasonal and annual changes in the content of Cu, Cd and As in the liver, skin and muscles of a carpenter from the

Bulgarian section of the Danube River. Scientific and applied contribution original for Bulgaria;

□ Excesses of Cu, Cd and As were found in the liver, skin and muscles of bream, bream and bream (except for Cu in the liver of bream) from the Danube River, Kudelin biotope. Confirmatory scientific-applied contribution with elements of originality;

□ The data on the circulation of As in the liver, skin and muscles of bream and bream, their parasites, waters and sediments from the Bulgarian section of the Danube River have been updated with the results of the Kudelin biotope. Confirmatory scientific-applied contribution with elements of originality;

□ It has been confirmed that the most prominent bioindicators are: liver of carp and ukleya for Cd content; bream liver for As content; *Contracaecum* sp. for Cd content; *Porcellio laevis* for As content. Confirmatory scientific-applied contribution with elements of originality;

□ The scientific literature has been enriched on the content of Cu, Cd and As in tissues and organs of bream, bream and bream, some parasites, as well as in waters and sediments of the freshwater ecosystem of the Danube River. Original scientific and applied contribution.

## **7. Critical notes, questions and recommendations.**

With the exception of some technical inaccuracies, I have no significant comments on the dissertation work and the abstract. However, the interpretation of the rich experimental data in a biochemical aspect could be a little more thorough. For example, the section "Results" could be "Results and discussion", where a discussion could find a place to touch on the biological role of copper, for example, and its involvement in some metalloproteins.

Given the volume and the high scientific value of the obtained data, I recommend their formation in a monograph related to the dissertation work.

## **8. Published articles and citations.**

The logical conclusion of any research is the publications related to it in prestigious scientific journals. According to the present dissertation, they are 2 nos. in Scientific Papers. Series D. Animal Science /refereed and indexed journals in world-renowned databases with scientific information/. In addition, the PhD student has 3 participations in 2 international scientific conferences – 16 and 17 International May Conference on Strategic Management – 2020 and 2021, in Bor, Serbia, indexed in

Web of Science. In all five publications, Petya Zaharieva is the lead author, which is a good certificate for her participation and contribution in the developments.

### **9. Evaluation of the autoref**

The abstract is a compressed version of each dissertation work and that of Petya Zaharieva has the necessary sections in the reference sequence and volume for similar publications. It is presented on 35 pages and reflects the main information in the dissertation. The exposed results, contributions, conclusions and recommendations are a valuable aid for the collegium in the scientific circles and those working in the field of ecology and the fish farming industry.

### **10. CONCLUSION:**

Based on the above, I believe that the presented dissertation meets the requirements of the RSARB. The topic and the biological species have been selected successfully. The scientific-experimental activity is organized and conducted with skill and competence. The results are obtained and processed with modern methods and equipment. The discussion is current and in tune with modern scientific achievements in the field. Formulated conclusions, contributions and recommendations are valuable for ecology and fish farming practice. The doctoral student, in addition to in-depth theoretical knowledge, also demonstrates enviable skills in analyzing and discussing the obtained results. In this aspect, Petya Zaharieva's training in the doctoral program has achieved the desired result and the materials presented in the procedure exceed the minimum national requirements. I highly appreciate the dissertation work, the abstract and the publications related to it and I firmly believe that the presented dissertation work meets the requirements of the ŽRASRB and the Regulations of the Agricultural University for its application. This gives me reason to rate it POSITIVELY and to recommend to the members of the respected Scientific Jury to also vote POSITIVELY for the awarding of the educational and scientific degree "Doctor" to Petya Georgieva Zaharieva in the Scientific specialty "Ecology and Ecosystem Preservation", professional direction 4.3. "Biological sciences", field of higher education 4. "Natural sciences, mathematics and informatics".

22.11.2022

Plovdiv

Signature: .....  
/Prof. Dr. Vasil Atanasov, DSc/